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Effect of glucose content on thermally cross-linked fibrous gelatin scaffolds for tissue engineering

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ABSTRACT

Thermally cross-linked glucose-containing electrospun gelatin meshes were studied as possible cell substrate materials. FTIR analysis was used to study the effect of glucose on cross-linking reactions. It was found that the presence of glucose increases the extent of cross-linking of fibrous gelatin scaffolds, which in return determines scaffold properties and their usability in tissue engineering applications. Easy to handle fabric-like scaffolds were obtained from blends containing up to 15% glucose. Maximum extent of cross-linking was reached at nearly 20% glucose content. Cross-linking effectively resulted in decreased solubility and increased resistance to enzymatic degradation. Preliminary short-term cell culture experiments indicate that such thermally cross-linked gelatin-glucose scaffolds are suitable for tissue engineering applications.

1. Introduction

Gelatin is a protein derived by hydrolysis from collagen, a naturally occurring protein most abundant in connective tissue [1,2]. Both collagen and gelatin have been considered as materials for tissue engineering scaffolds and share many advantages (biocompatibility, non-toxicity) as well as disadvantages (nonhomogeneous composition and inconsistent physicochemical properties) [3], but gelatin has been proven to be less immunogenic [1] and is easier to process.

In order to mimic the structural component of extracellular matrix (ECM) that is built from protein micro- and nanoscale fibres in natural tissue, the artificial substrate should also be presented to the cells in the shape of a fibrous polymeric network. Several methods have been considered for this purpose including phase separation, self-assembly, surface patterning, wetspinning, biospinning, interfacial complexation, microfluidic spinning, meltspinning and electrospinning [4–6]. The latter method is a well-established, simple, cost-effective and flexible technique that takes advantage of electrostatic forces to produce polymer fibres [5]. It has become widely accepted in recent years that conventionally electrospun meshes cannot support cell penetration into deeper layers due to small pore size [6]. Nevertheless, electrospinning has the advantage of producing structural features similar to ECM, which has been shown to result in different cell morphologies compared to cells grown on smooth substrate [7].

Porous gelatin scaffolds are known to be suitable for fibroblast culture [3,8], although some critical issues need to be addressed in order to develop materials with structural features required for tissue engineering applications. Prepared from water-insoluble collagen, gelatin undergoes structural changes during preparation and is a mixture of water-soluble proteins. This causes the properties of untreated scaffolds to be different from those of natural collagen-based ECM. Since cells are seeded on the scaffolds in aqueous environment, fibrous gelatin scaffolds must be made insoluble in water to preserve structure and provide mechanical support to cells during tissue formation.

Cross-linking decreases solubility by creating chemical bonds between polymer chains. In addition, cross-linking has been shown to enhance mechanical properties of fibrous gelatin-based materials [9]. Stiffness of artificial substrate materials is known to influence cell

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growth, proliferation and differentiation [10,11]. Therefore, cross-linking can be used to make gelatin fibres insoluble and to tune their mechanical properties. Cross-linking of gelatin fibres can be carried out by various chemical methods (using dialdehydes, phenolic compounds, genipin etc. [12–15]), enzymatic [16] and physical [1,17] means or a combination of these. However, a large proportion of chemical cross-linkers are more or less toxic and thus potentially dangerous in the field of tissue engineering. Gelatin scaffolds can also be cross-linked thermally [18], but adding cross-linking agents can enhance scaffold properties even further.

The properties of gelatin fibres are dependent on the extent of cross-linking. The presence of sugars can alter the conformation and interactions of proteins, enables the Maillard reaction to take place and has an effect on the cross-linking process [19–21]. Thus, adding sugars to gelatin scaffolds could help us to enhance fibre properties by controlling the extent of cross-linking. This has brought about the need to study the effect of the concentration of sugars on the extent of cross-linking of gelatin scaffolds.

The purpose of this research was to investigate the effect of glucose concentration on thermally induced cross-linking in electrospun gelatin fibres. Scaffolds were prepared from both type A and type B gelatin and used in cell culture experiments. Fourier transform infrared spectroscopy (FTIR) was used to monitor the cross-linking process. Fibre morphology and structure of obtained scaffolds were analysed by scanning electron microscopy (SEM). Scaffolds with different glucose concentrations were tested for possible leaching of glucose and enzyme mediated degradation. In order to evaluate the ability to support cell growth and proliferation, fibroblasts were seeded on scaffolds with different glucose content and analysed after culturing for one day (24 h) and seven days.

2. Materials and methods

2.1. Preparation of fibrous scaffolds

Gelatin type A from porcine skin, gelatin type B from bovine skin, D-(+)-glucose and glacial acetic acid were purchased from Sigma-Aldrich. Gelatin was mixed with glucose at different ratios. The mixtures were dissolved in 10 M aqueous acetic acid solution at about 40 °C by vigorous stirring to obtain solutions containing 25% gelatin. Fibrous scaffolds were prepared from these solutions by electrospinning. A syringe containing gelatin solution was mounted on a New Era Pump Systems NE-511 pump operating at speeds between 6 and 8 μl/min. High voltage between 17 and 18 kV was applied to metallic syringe needle using Heinzinger LNC 30,000 high voltage power supply. A grounded target was placed 14 cm away from the needle tip. Fibrous meshes were collected from the target after electrospinning and stored for further treatment.

2.2. Thermal cross-linking

Gelatin scaffolds were cross-linked thermally by placing them in an oven for 3 h. In order to avoid thermal degradation of gelatin while ensuring proper cross-linking [17,18] and to operate above melting point and caramelization temperature of glucose, cross-linking was carried out at 170–175 °C. Additionally, pieces of some scaffolds were removed from the oven after various times between 5 min and 3 h and analysed to monitor the cross-linking process.

2.3. Scaffold structure and fibre morphology

SEM (Tescan Vega) was used to confirm the fibrous structure of the scaffolds before and after cross-linking. Samples were dried using Leica EM CPD300 critical point drier and covered with 5 nm layer of gold using Polaron SC7640 sputter coater. Several images of every scaffold were recorded at 21,000 times magnification. Fibre diameters were measured from 3 representative 21,000 times magnified images.

2.4. FTIR analysis

Scaffolds were analysed by FTIR before and after cross-linking using Bruker Vertex 70 spectrometer equipped with an attenuated total reflection (ATR) accessory (diamond ATR-crystal). Prior to recording spectra, all meshes were dried in a critical point drier (Leica EM CPD300). ATR spectra were recorded between 400 and 4000 cm^{-1}. Recorded spectra were converted to absorbance spectra after baseline correction using OPUS software. All spectra were normalized to a constant penetration depth.

Absolute values of recorded absorbances were not reproducible due to fibrous scaffold structure and small fibre diameter, which did not allow achieving perfect contact between scaffolds and ATR crystal. To be able to reliably compare infrared spectra of scaffolds with different compositions before and after cross-linking, relative absorbances (RA) were calculated by dividing absorbance of the peak in question by the sum of absorbances of all peaks detected in this particular spectrum. Such comparison is made easier by the fact that peaks at 20 different wavenumbers were detected and all of them were present in every scaffold analysed. RA-s were very sensitive to practically all stages of scaffold preparation and analysis, but were found to be reproducible upon careful handling. Measurement errors were insignificant compared to variability of calculated RA-s, probably because gelatin powders used were not completely homogeneous. Errors of RA-s were therefore determined by calculating the biggest difference in RA-s of samples containing the same amount of glucose. In spite of difficulty in achieving reproducibility, the tendencies discussed below were always the same. Thus, it was found that calculating and comparing RA-s is a suitable way to analyse and compare glucose-containing gelatin scaffolds.

2.5. Cell culture and SEM images of cells on scaffolds

All procedures considered in this work were carried out in accordance with the ethical standards of the Research Ethics Committee of the University of Tartu. A written informed consent was obtained from all patients. The patients were recruited from among elective patients present at the Department of Pediatric Surgery, Tallinn Children's Hospital. Cell culture experiments were carried out according to previously described protocol [22] with the following modifications. Primary fibroblasts (2700 cells/cm^2) were seeded onto glass cover slips covered with fibrous scaffolds. Scaffolds with fibroblasts were collected 24 h and seven days after seeding and fixed with Karnovsky buffer. The samples were dehydrated through alcohol gradient, dried using a critical point drier (Leica EM CPD300) and analysed by SEM.

2.6. In vitro degradation of scaffolds

The biological stability of the scaffolds was evaluated by exposing them to collagenase type II (2 Units/ml; PAA), collagenase type IV (160 Units/ml; Gibco) and 0.25% trypsin (Invitrogen) for 24 h. Degradation tests were performed at 37 °C in a horizontal shaker.

2.7. Glucose measurements

Quantitative glucose oxidase and peroxidase kit (Spinreact) was used according to the manufacturer's guidelines in order to detect possible leaching of glucose from the scaffolds. The forming hydrogen peroxide binds to chromogenic oxygen acceptor (phenol) and indicates the amount of glucose. Scaffolds were soaked in phenol red free medium (DMEM — Dulbecco's Modified Eagle Medium) for 8 h at 37 °C. Incubated medium was removed for glucose measurements and replaced with fresh medium. Glucose measurements were repeated after incubating the scaffolds in fresh medium for 8 h. DMEM was removed before glucose detection. Spectrophotometer (Tecan) with Magellan (Tecan Group Ltd., Switzerland) software was used at

505 nm wavelength to detect the intensity of red quinoneimine dye, which is proportional to glucose concentration in the samples.

2.8. Quantification of viable cells

CellTiter-Glo® Luminescent Cell Viability Assay® (Promega) was used to quantify the number of viable cells grown on the scaffolds according to the manufacturer's protocol. Cell viability tests were performed 16 h and 7 days after seeding. The readings were detected using Tecan Infinite M200Pro luminometer.

2.9. Protein mass spectrometry (MS)

Protein MS was performed using LTQ Orbitrap XL (Thermo Fisher) mass spectrometer. MaxQuant 1.4.0.8 software was used for protein identification according to the manufacturer's protocol. Samples were incubated overnight at 4 °C and purified with StageTips (C18) after in-solution digestion with proteases lysC and trypsin in 8 M urea. Nanoliquid chromatography–tandem MS analysis was performed using acetonitrile 8–40% gradient and 0.5% acetic acid.

3. Results

3.1. Preparation of fibrous scaffolds

Gelatin can be electrospun from acetic acid or its water solutions, but fibres are produced only when the concentration of acetic acid is sufficiently high [23]. Mixtures of both type A and type B gelatin and glucose were found to be electrospinnable from 5 M and more concentrated aqueous acetic acid solutions, but the suitable range of electrospinning parameters became narrower at lower concentrations. Glucose concentration had little effect on the suitable range of electrospinning parameters, which seemed to be determined mostly by gelatin and acetic acid concentrations. 10 M acetic acid solution allowed problem-free electrospinning of all solutions used in this work. Scaffolds containing up to 50% glucose were electrospun successfully. However, easy to handle fabric-like scaffolds were produced at up to 15% glucose content. At higher glucose concentrations the scaffolds became stiff, brittle and broke easily after thermal cross-linking. Scaffolds containing over 30% glucose were not usable at all and were therefore excluded from the following stages of this study. Before cross-linking, the scaffolds were water-soluble. After cross-linking, the scaffolds became insoluble in boiling water, glacial acetic acid and cell culture media.

3.2. SEM

Fibrous structure of gelatin scaffolds was confirmed by SEM (Fig. 1). Fibres were smooth and uniformly structured. At certain glucose content, the average fibre diameters varied almost up to two times depending on electrospinning conditions (voltage, distance between needle tip and grounded collector, relative air humidity etc.). For example, the average fibre diameters of pure gelatin scaffolds prepared using different electrospinning parameters varied between 280 and 575 nm. Fibre diameters decreased until glucose content reached about 5%, then started to increase again as glucose concentration became higher. The dropping of fibre diameters is probably associated with the effect of glucose, whereas the increase of fibre size can be explained by the increasing viscosity of electrospinning solution and the fact that while every electrospinning solution contained 25% gelatin, it contained a smaller and smaller percentage of solvent as glucose was added to the solution.

Comparison of SEM images (Fig. 1) revealed no significant structural differences (neglecting fibre diameters) between samples with different glucose concentrations. Likewise, SEM images of scaffolds before and after cross-linking were almost identical.

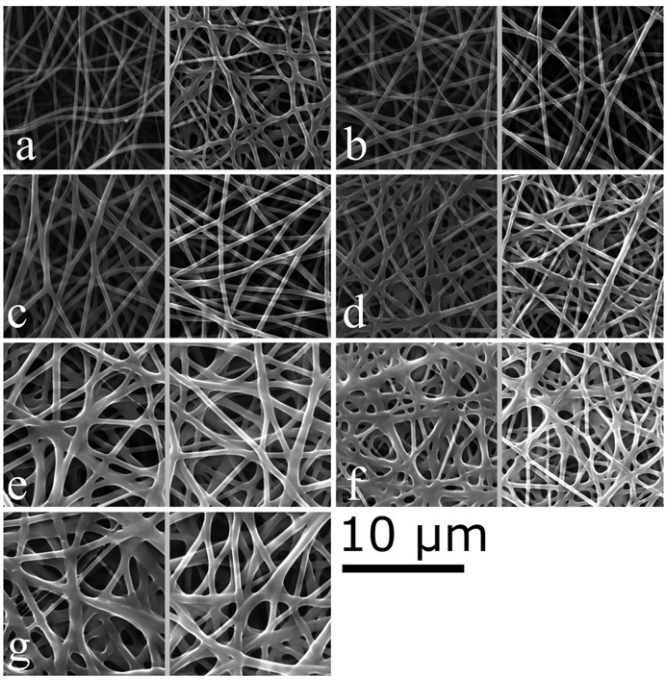

Fig. 1. SEM images (21,000 times magnification) of gelatin scaffolds with various glucose content before (left) and after (right) cross-linking: a) 0% b) 5.5% c) 9.9% d) 15.1% e) 20.4% f) 25. % g) 30.2% glucose.

3.3. FTIR analysis

FTIR spectroscopy was used to compare gelatin scaffolds with different glucose content, to analyse the effect of glucose on structural changes during thermal cross-linking at 170–175 °C and to determine the optimal time of exposure to this temperature, as well as to evaluate the extent of cross-linking. There were very little differences between FTIR spectra of type A and type B gelatin scaffolds (a little bit higher or lower peak intensities at certain wavelengths) and the tendencies discussed below were identical, so we only present analysis of type A gelatin in this subdivision.

For all spectra, peaks were detected at 3285, 3077, 2939, 2879, 1657, 1640, 1631, 1562, 1547, 1535, 1452, 1405, 1336, 1240, 1203, 1163, 1081, 1035, 921 and 629 cm^{-1} (Fig. 2). Due to the complex structure of the scaffolds, many peaks of gelatin and glucose overlap, making it harder to be sure which changes in spectra during cross-linking are brought about by gelatin–glucose interactions and which by gelatin–gelatin or glucose–glucose interactions. The peaks detected between 1700 and 2700 cm^{-1} were ignored, because they had low intensity and did not provide valuable information at this stage of research.

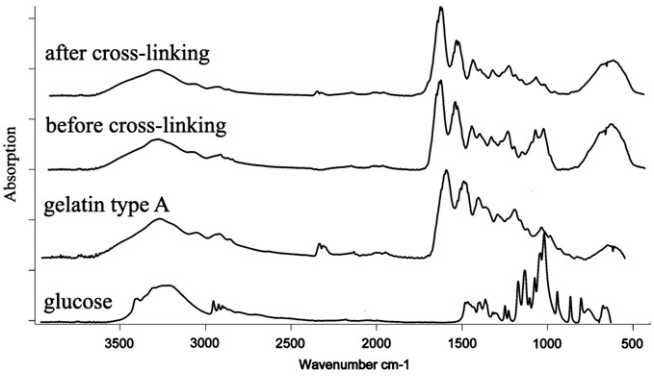

Fig. 2. FTIR spectra of glucose, type A gelatin and electrospun scaffolds containing 15.1% glucose before and after cross-linking.

Little changes in RA were detected for peaks at 3285, 3077 cm^{-1} (representing mainly OH and NH vibrations [9,18]), 2939 and 2879 cm^{-1} (representing mainly CH_2 asymmetric and symmetric vibrations respectively), 1452, 1405, 1336, 1203 and 1163 cm^{-1} (representing different in plane vibrations [24]) and 1240 cm^{-1} (amide III [9,25]). Contrary to these, the amide I (peaks at 1657, 1640 and 1631 cm^{-1}, mainly C═O vibrations) and amide II (peaks at 1562, 1547 and 1535 cm^{-1}, mainly NH bending) [9,18,25,26] bands as well as peaks at 1081, 1035 and 921 cm^{-1} (mainly CO vibrations overlapping with over vibrations in glucose [24]) went through considerable changes in RA upon the addition of glucose and during cross-linking, as is shown later on.

Firstly, IR spectra of scaffolds with different glucose content before cross-linking were analysed. Increasing glucose content in the fibres from 0% to 30% led to considerable increase in RA (Table 1) of peaks at 1081 cm^{-1}, 1035 cm^{-1} and 921 cm^{-1}. At the same time, RA of amide I and amide II bands decreased. These are the peaks by which we can best distinguish between changes in gelatin and changes in glucose during cross-linking.

Next, spectral changes during cross-linking were analysed. In order to find the optimal time of exposure to 170–175 °C, the cross-linking process was monitored by removing pieces of scaffolds from the oven after various times, analysing these by FTIR and comparing resulting spectra. It was found, as expected, that changes in RA during thermal cross-linking are exponential (Fig. 3a). Considerable changes were detected during the first hour of cross-linking. After 2 h of thermal treatment, very little further changes in RA were detected with an average of less than 0.1% change between 2 and 3 h as opposed to an average of about 5% change in RA during the first 5 min of thermal treatment. It was concluded that the optimal period of thermal cross-linking at 170–175 °C is about 3 h.

Next, spectra of gelatin scaffolds with 0–30% glucose content before and after cross-linking were compared to analyse structural changes and determine the extent of cross-linking. Major changes in RA were seen at 1647, 1640, 1631, 1081 and 1035 cm^{-1}. Changes in RA (Fig. 3b) were calculated by subtracting the RA of a given peak before cross-linking from the RA of the same peak after cross-linking.

The interpretation of spectral changes is made harder by the number and complexity of reactions going on during cross-linking. Whereas spectral changes in pure gelatin scaffolds are limited, it was observed that scaffolds with higher glucose content undergo rapid changes during cross-linking, which indicates that the majority of reactions going on during cross-linking are directly caused by the presence of glucose.

By far the biggest change in RA during cross-linking of scaffolds was the increase of RA of the amide I band, coupled with the decrease of peaks at 1081 and 1035 cm^{-1} associated with C–O vibrations mainly in glucose. One of the major reactions occurring in gelatin–glucose composites at high temperatures is probably the Maillard reaction, the reaction between carbonyl group of glucose and amino group of gelatin (mainly the ε-amino group of lysine, but also the α-amino groups of terminal amino acids [27]). Certain amount of caramelization may also occur in glucose-containing scaffolds, but this is probably less important until the maximum extent of cross-linking is reached.

This brings up the question of the amount of glucose at which gelatin is cross-linked to maximum extent that can be achieved using glucose as cross-linking agent. The extent of gelatin cross-linking is often evaluated by changes in free ε-amino group concentration [28], although it has been suggested that at high temperatures a cross-linking mechanism without an amino group involvement occurs [29]. The increase in RA at 1657, 1640 and 1631 cm^{-1} was strongest for scaffolds containing about 20% glucose, while RA at 1081 and 1035 cm^{-1} decreased further still when scaffolds contained more glucose. This suggests that some other reaction, perhaps caramelization, will become more dominant at over 20% glucose content.

IR spectra also give some valuable information about secondary structure of proteins. Fibres electrospun from water solution have been found to have significant triple helix content, while electrospinning gelatin from glacial acetic acid resulted no triple helix content [30]. No changes in the shape of the amide I band were detected after cross-linking, which suggests that the triple helix structure typical of proteins is already broken during preparation of electrospinning solution.

3.4. Assessment of biological properties

3.4.1. Glucose measurements

In order to demonstrate biocompatibility of the material, primary skin fibroblasts were seeded onto fibrous gelatin scaffolds prepared using various glucose concentrations. It was noticed during preliminary experiments that glucose concentration in the cell culture media rose after incubating the scaffolds in it, which could influence the growth characteristics of the cells. In order to investigate the reasons for this, the scaffolds were incubated in DMEM and glucose concentration in the culture medium was measured. It was found that after such incubation glucose concentration increased from 7% to 29% (Fig. 4) compared to fresh DMEM, which normally contains 450 mg/dl glucose. The change in glucose levels for type B gelatin scaffolds was somewhat larger than for type A gelatin scaffolds (22.1% and 15.3% respectively). A probable explanation is that the degree of swelling is somewhat different for type A and type B gelatin. When the culture medium was replaced with fresh one, the glucose level in the medium did not increase. The amount of excess glucose in the medium did not correlate with glucose concentration present in gelatin blends. Furthermore, glucose levels also increased in case of pure gelatin scaffolds. It was concluded that this effect can be attributed to water absorption during swelling of the scaffolds. Considering this, all the experiments were performed with matrices pre-incubated in the growth medium.

3.4.2. Cell proliferation

Next, the viability and proliferation of the fibroblasts seeded onto the scaffolds were studied using a luminometer-based cell viability assay (Fig. 5). After 16 h of culturing, the number of cells attached to the scaffolds was largely the same for all glucose concentrations. Seven days after seeding the cells had started to proliferate and the number of fibroblasts increased remarkably. Notably, at higher glucose concentrations (25% and 30%) the number of viable cells was significantly lower. However, the differences between the total number of

Table 1
Effect of glucose content on RA before cross-linking.

% glucose cm^{-1}	0% RA	5.49% RA	9.92% RA	15.09% RA	20.42% RA	25.5% RA	30.15% RA
1657	9.6	8.4	8.5	9.1	7.0	7.4	7.7
1640	11.7	10.3	10.5	10.6	8.5	9.2	9.2
1631	11.9	10.8	11.0	10.7	9.0	9.8	9.6
1562	7.1	6.7	6.7	6.7	6.0	5.9	5.7
1547	8.7	8.2	8.2	8.1	7.3	7.3	7.0
1535	8.5	7.8	7.8	7.6	7.0	6.9	6.6
1081	2.7	3.7	4.1	4.8	5.4	5.6	6.2
1035	1.8	3.4	4.1	4.9	6.1	6.8	7.7

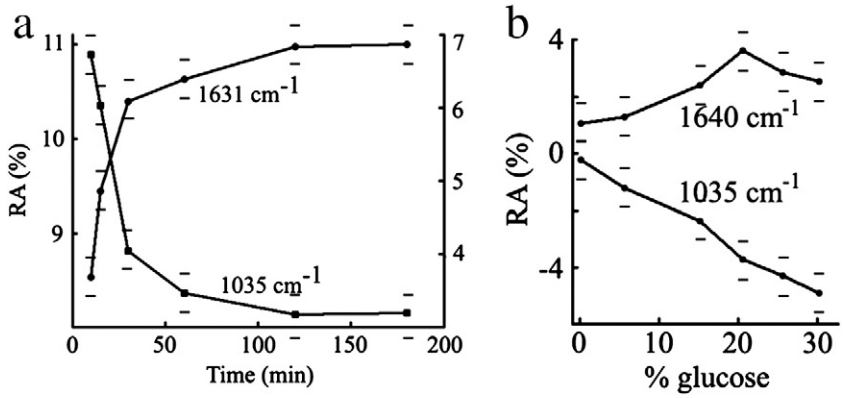

Fig. 3. a. Changes in RA during cross-linking; 19.9% glucose content. b. Effect of glucose content on changes of RA.

cells grown on type A and type B gelatin scaffolds containing the same amount of glucose were marginal. The number of cells started to decrease notably at 10% (type B gelatin) and at 25% (type A gelatin) glucose concentrations.

3.4.3. Cell morphology

SEM images confirmed the results of proliferation tests. The morphological differences between cells on scaffolds with different glucose content were clearly evident and were caused by variations in scaffold porosity, stiffness, fibre diameter, fibre orientation and degree of swelling. Cells grown on scaffolds with larger pores formed more dendrites to connect to the surrounding scaffold (Fig. 6a) than cells grown on flat surface or meshes with narrower pores (Fig. 6c, d). Cells on samples with thinner fibres and higher mesh density appeared to have a flat morphology similar to the cells grown on 2D surface like plastic or glass (Fig. 6d), suggesting that these scaffolds fail to effectively present structural cues to the cells. Certain directional orientation of cells grown on substrates with larger and more loosely packed fibres was evident, especially in regions where parallel ordering of fibres was observed (Fig. 6b).

3.4.4. Biological degradation

The biological stability test of cross-linked gelatin scaffolds using digestion with collagenases (type II, IV) and trypsin showed that scaffolds with higher glucose content (25% and 30%) were resistant to digestion with all three types of enzymes, as scaffolds maintained their pre-treatment shape. Scaffolds containing 20% glucose were partially degraded. Matrices containing 15% and less glucose were totally dissolved in collagenase and trypsin solution and no undigested scaffold fragments could be observed under optical microscope.

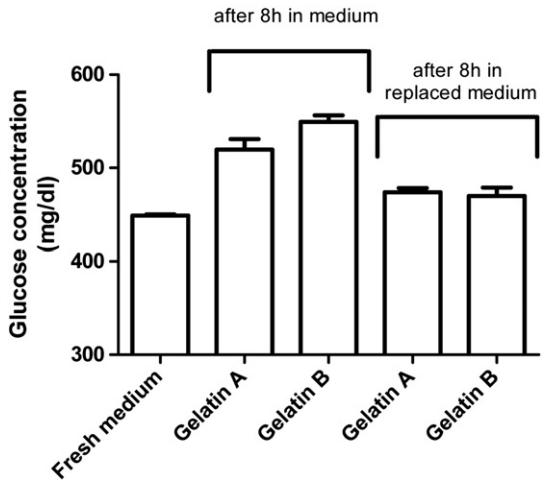

Fig. 4. Change in glucose concentration during swelling.

3.4.5. Protein analysis

Mass spectrometry (MS) analysis revealed that protein content of type A and type B gelatin used in this work was significantly different. It was noticed that type A gelatin used in this work was a more complex mixture of various proteins than type B gelatin and in addition to compounds originating from skin, there was a large number of proteins found in blood, bones and other connective tissues (Table 2). Meanwhile the total amount of protein (per milligramme of powder) seemed to be a bit lower in case of type B gelatin compared to type A gelatin.

4. Discussion

It was demonstrated that glucose can be used to increase the extent of cross-linking in gelatin nanofibres. Gelatin nanofibres can be cross-linked by thermal treatment alone, but combining thermal treatment with cross-linking by glucose allows us to control the extent of cross-linking to a large degree by varying glucose content in the fibres.

The results of FTIR studies (Section 3.3) and biological stability test (Section 3.4) suggest that glucose is an effective cross-linking agent for thermal cross-linking of gelatin scaffolds. In addition to being non-toxic, it also reacts and polymerizes readily at high temperatures. Therefore, it is not necessary to clean the scaffold of residual cross-linking agent, although pre-treatment of scaffolds is necessary due to swelling (Section 3.4).

Evaluating the extent of cross-linking by analysing FTIR spectra proved to be effective. However, it must be noted that the absolute values of the determined extent of cross-linking are dependent on the method itself and the results are hard to compare to those obtained by other methods, for example near-infrared spectrophotometry [31] and use of chemical assays [32].

The extent of glucose-induced cross-linking increases up to about 20% glucose content. Both cell culture experiments, biological stability test (Section 3.4) and observations made during scaffold preparation (Section 3.1) indicate that scaffolds containing up to 15% glucose can be considered for tissue engineering applications, firstly because fabric-like easy to handle scaffolds were produced at 0–15% glucose content, secondly because the number of cells on scaffolds started to drop at higher glucose concentrations, and thirdly because at over 15% glucose content the scaffolds become resistant to enzymatic digestion, which is undesirable for tissue engineering applications.

Glucose, albeit an essential energy source, is toxic to cells when its concentration exceeds a certain value. This phenomenon is observable in case of diabetes, when long-term high blood glucose level can cause abnormal collagen cross-linking and thereby affect structure, mechanical properties and functioning of tissues [33]. Therefore, it is necessary to keep glucose levels in physiologically suitable range during cell culture.

It was found that cell growth characteristics varied on scaffolds prepared using different amounts of glucose (Fig. 5). Proliferation of

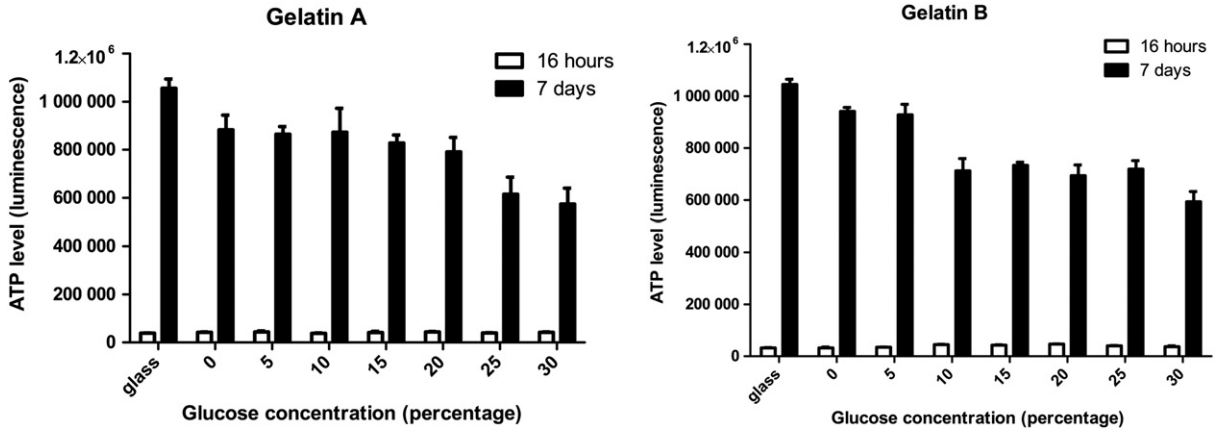

Fig. 5. Dependence of ATP levels on glucose concentration 16 h and 7 days after seeding. ATP level is proportional to the number of cells.

fibroblasts started to decrease from certain glucose content. Scaffolds prepared using 20% or higher glucose content became partially or fully resistant to enzymes responsible for degradation of extracellular matrix in the body. These results, combined with data obtained from FTIR analysis, suggest that the maximum extent of cross-linking was achieved at approximately 20% glucose content. If used in tissue engineering or wound healing applications, however, the scaffold should degrade over time.

Although gelatin has been regarded to mainly consist of type I collagen (composing of two alpha 1 and one alpha 2 chains) the content depends on the exact extraction method as well as the origin of raw material. Previous studies have shown that under the same conditions collagen type I had higher cross-linking capacity compared to mixtures of collagen types I and III. The reason for this phenomenon is related to the dissimilarities in primary structures and molecular organization of different types of collagen [34]. Protein analysis indicated remarkable difference between type A and type B gelatin used in this work. Both high rate of additives and different protein profiles might have affected the cross-linking process, leading to differences in scaffold properties and cell growth.

5. Conclusions

The possibility of using glucose as cross-linking agent in electrospun fibrous gelatin scaffolds for tissue-engineering applications was explored. Scaffolds were prepared from gelatin–glucose blends using only natural, non-toxic substances and simple, cost-effective methods. FTIR relative absorbance studies indicate that increasing glucose content in

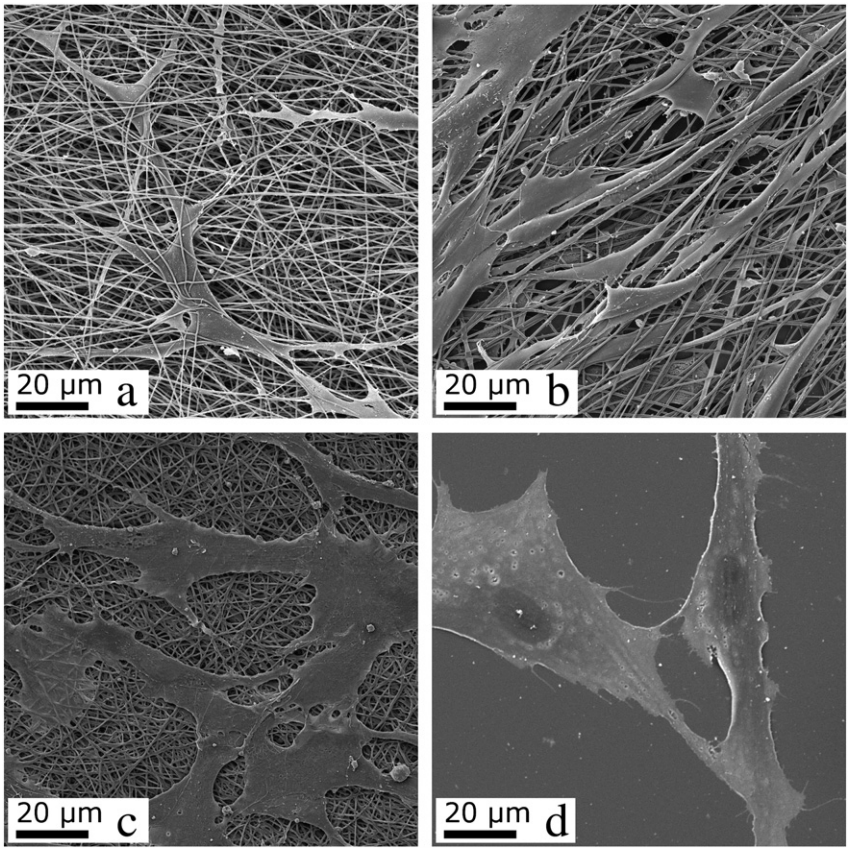

Fig. 6. Morphology of fibroblasts grown on scaffolds with different porosity, fibre density, orientation and diameter (2500 times magnification). a) 5% glucose b) 10% glucose c) 30% glucose d) glass.

Table 2
MS results. Protein profiles of gelatin type A and type B powders. Peak intensity corresponds to the relative abundance ratio of proteins.

Gelatin, type A		Gelatin, type B	
Protein name	Intensity	Protein name	Intensity
Collagen, type I, alpha 2	7.02E + 09	Collagen, type I, alpha 1	1.95E + 09
Collagen, type I, alpha 1	2.98E + 09	Collagen, type I, alpha 2	1.35E + 09
Fragments of collagen, type I, alpha 1 and alpha 2	1.23E + 09	Collagen, type III, alpha 1	1.33E + 08
Collagen, type III, alpha 1	7.14E + 08	Decorin	2.69E + 06
Collagen, type V, alpha 2	4.93E + 08	Myosin, heavy chain 7, cardiac muscle, beta	1.61E + 06
Albumin	1.02E + 08	Albumin	1.57E + 06
Decorin	4.66E + 07		
Collagen, type V, alpha 1	4.05E + 07		
Lumican	3.27E + 07		
Orosomucoid	3.17E + 07		
Transferrin	2.11E + 07		
Osteoglycin	1.77E + 07		
Transthyretin	1.34E + 07		
Serpin peptidase inhibitor, clade A, member 3-3	1.12E + 07		
Collagen, type V, alpha 3	2.44E + 06		
Fragments of serpin peptidase inhibitor, clade A, member 3-3	9.42E + 05		
Chitinase, acidic	8.91E + 05		
Serpin peptidase inhibitor, clade A, member 3-2	3.17E + 05		

gelatin scaffolds efficiently increases the extent of cross-linking up to about 20% glucose content. Monitoring the cross-linking process showed that the optimal period of cross-linking glucose-containing gelatin fibres at 170–175 °C is about 3 h. At about 20% glucose content the fibres become resistant to enzymatic digestion, a process responsible for physiological degradation of extracellular matrix in the body, indicating that up to 20% glucose concentrations can be used to obtain bioresorbable scaffolds. Fabric-like, easy to handle scaffolds were produced at up to 15% glucose content. Obtained cross-linked scaffolds supported fibroblast growth and cell–scaffold interactions.

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